0022-202X/84/8301S-0067s$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 83:067s-071s, 1984

Vol. 83, No. 1 Supplement
Printed in U.S.A.

Herpes Simplex Virus Type 2 Establishes Latency in the Mouse Footpad and in the Sensory Ganglia

J. H. SUBAK-SHARPE, B.SC., PH.D., F.R.S.(E.), S. A. AL-SAADI, B.V.M.S., M.SC., PH.D.,* AND G. B. CLEMENTS, M.A., M.B., B.CHIR., PH.D.

Department of Virology, University of Glasgow, Glasgow, Scotland

Three mouse strains as well as *wt* and 7 *ts* mutants of herpes simplex virus (HSV) type 2 (strain HG52) have been used to investigate latency. The mice were inoculated in the right rear footpad. Virus reactivation following explanation and culture of the dorsal root ganglia and the footpad was scored. The results show that: (1) HSV-2 can be maintained in the mouse footpad in a state indistinguishable from latency; 2) virus gene functions necessary for latency can be identified by the use of *ts* mutants; and (3) mouse strains differ in their ability to support latent infection. An infectious center assay was used to quantitate virus reactivation from dissociated dorsal root ganglia. HSV-1 strain 17 *wt* spread after inoculation at doses of $\geq 5.0 \times 10^5$ plaque-forming units (pfu), producing latency also in contralateral ganglia but with lower efficiency.

The great majority of viruses invade and productively infect the natural host's susceptible cells; this triggers an immune response and as a result the virus is usually eliminated from the host's body. Following recovery the individual is then usually protected against a further infection by the same or an antigenically similar virus. At all times, the virus population is generated and maintained by successive cycles of productive infection. The viral genomes are exposed to immediate selection at every infectious cycle; only progeny that for one reason or another survive these multiple internal and external selective pressures contribute to the composition of the next generation's gene pool. Frequent genetic changes of the virus population can result—an extreme illustration is provided by the constantly changing influenza A virus. A large population of potential hosts is needed for such virus to be maintained endemic in the region; moreover, the population of viral genomes forming the gene pool varies with availability of susceptible individuals.

However, the herpes viruses have evolved an additional strategy: the facility to produce latency which stabilizes the composition of the viral gene pool over longer periods of time. This strategy is accomplished by the virus succeeding in establishing a state of latency in privileged host cells, in the case of HSV neurons, from which it retains the capability of reactivating periodically to produce repeated productive localized infections which may serve as sources of virus and cause primary infections in other individuals. Opportunity for selectively imposed genetic change occurs at every round of viral genome replication. When the virus is latent, this opportunity does not recur every few hours or days as is the case during normal infectious cycles, but only at infrequent intervals (months or years) when reactivation occurs. Thus herpes viruses have retained all the potential advantages of the productive infectious cycle, with the added boon that progeny of the same genome are repeatedly exposed to selection over many years. The population of viral genomes forming the gene pool is at all times large and stable and continues to evolve with the host.

The term *latency* as used here implies the ability of the viral genome to infect and remain essentially dormant and unexposed to selection in certain host cells—it remains "frozen" for very prolonged periods of time. At *all* times latent genomes retain the potential to reactivate, producing fully viable progeny virus particles that can infect other host cells and hence also new hosts. A latent virus genome survives in a privileged site and is not detected by, or exposed to, the host's immune defenses. In the case of HSV, it has become widely accepted that the viral genome, following primary infection at the periphery, establishes latency in nervous tissue, particularly in neuronal cell bodies of the sensory ganglia anatomically related to the affected sites [1]. However, evidence is beginning to accumulate that there may be additional cell types in which HSV can establish latency.

Humans are the only natural host of herpes simplex virus (HSV), type 1 and type 2. HSV-1, often referred to as facial herpes, has been shown to be able to become latent in the trigeminal [2], superior cervical, and vagus ganglia [3]. On reactivation, HSV-1 may initiate lesions on the lips, mouth, eye, or less commonly, on other sites in the dermatome innervated by the respective sensory ganglion, and infectious virus can be isolated from these overt lesions. At times, following reactivation, HSV is shed in the absence of detectable lesions [4]. HSV-1 has also, with increasing frequency, been isolated from the genital regions. HSV-2 has a predilection for infecting the genital regions of men and women and is frequently referred to as herpes genitalis.

Herpes virus infecting the genital regions has been shown to go latent in the sacral ganglia [5] and there is, in addition, one recent report of successful reactivation from explanted utero-sacral ligaments in which occasional parasympathetic ganglia could be demonstrated [6]. Reactivation usually leads to lesions on the cervix, external genitalia, the anal region, and the buttocks.

Latency is postulated to become established by virus genomes travelling up the axons of sensory neurons innervating the region of the neuronal cell body in the ganglion and entering a dormant state there. The molecular biology of latency has still to be elucidated. Occasionally in response to a variety of stimuli, either external or initiating in the host's body, the latent genomes are caused to reactivate with the production of infectious virus. Here again the molecular events remain obscure. The progeny viral genomes are thought to travel via the axoplasm by axonal transport to the periphery—whether as subviral or fully formed virus particles is not established—and are there able to cause productive infection and local lesions. Whether a neuron in which reactivation occurs is or is not destroyed in the process is still controversial. Normally, cells that are productively infected are destroyed as a consequence of producing and releasing virus progeny.

Many attempts to isolate latent herpes virus established that

* On leave from the University of Baghdad.
Reprint requests to: Dr. J. H. Subak-Sharpe, Department of Virology, University of Glasgow, Glasgow G11 5JR, Scotland.

Abbreviations:
DRG: dorsal root ganglia
FP: footpad
HSV: herpes simplex virus
pfu: plaque-forming units

the virus could be reactivated only by explantation of ganglionic tissue and then only provided that the tissue was explanted within a few hours after death [7]. It was not possible to reactivate virus by explanting tissue from the peripheral sites (e.g., skin), in which recurrence normally took place, unless an overt lesion was present [8]. In an extensive study [3,9,10], a variety of ganglia were explanted within 24 h of death from many individuals who had died of trauma. Reactivated virus was subjected to restriction endonuclease analysis and the findings established that (a) the HSV genome reactivated from every individual could be distinguished from that obtained from any other, (b) where several different isolations were made from explants of the same ganglion, they produced indistinguishable virus genomes, (c) virus isolates from different ganglia of the same individual always possessed indistinguishable genomes, and (d) all isolates from these sensory ganglia were HSV-1. These findings strongly suggest that HSV, at the time of its primary infection, had spread and established latency in different neurons of one or more ganglia. Latency has subsequently been maintained without the type of evolutionarily acquired small changes taking place that would be expected if the viral genomes were continuously replicating throughout the latent period [11]. Following the initial establishment of HSV latency, it appears to become much more difficult for a subsequently infecting HSV strain to establish latency, even though many episodes of reinfection must occur during the lifetime of that individual. However, patients infected with both types of HSV are not uncommon, and individuals presenting with recurrent HSV-1 in the facial regions and HSV-2 in the genital regions have been reported. In most of these cases, rigorous proof that the virus was truly latent in the sensory ganglia is not available but this can be assumed.

Humans are the natural host for HSV, but laboratory animals can be infected and provide several model systems for the study of latency [1, 7]. Two model systems particularly relevant to our study use the guinea pig footpad and the mouse ear. Scriba studied infection of the guinea pig and reported that the virus produces latency in the dorsal root ganglia (DRG) following injection into the footpad (FP) of the hind limb, but infectious virus could subsequently be isolated at most times from the site of inoculation [12–14]. Several different mouse models have been established and in all of them, latent virus can be reactivated by explantation of the relevant nervous tissue. In recent years, Hill and colleagues have extensively studied a mouse ear model in which viral reactivation occurs spontaneously at low frequency leading to microscopic lesions in the skin [15]. The frequency of reactivation can be increased following stimulation of the ear skin [16–18]. Extensive earlier attempts to reactivate virus succeeded only in reactivating HSV from sensory ganglia, brain tissue, and the adrenal gland of the mouse [7].

Early collaborative experiments between Stevens' and Subak-Sharpe's groups provided the first qualitative evidence that specific HSV-1 *ts* mutants affect latency [19,20]. Our labortory has now undertaken a more extensive genetic study of HSV-2 latency using 7 *ts* mutants from different complementation groups [21–23] and 3 different strains of mice. The aims were threefold:

1. To establish whether genetically distinct mouse strains infected identically exhibit latency at different frequencies.
2. To reinvestigate whether virus can be reactivated only from the DRG or also from explanted tissue from the site of inoculation (FP) which is not known to contain neuronal cell bodies.
3. To identify HSV-2 *ts* mutations that alter the ability of the virus to establish latency in the DRG of the mouse.

Our results demonstrate:

1. Mouse host strain differences;
2. HSV-2 *ts* mutant related effects;
3. Maintenance of HSV-2 in the mouse footpad in a state indistinguishable by present criteria from latency.

The work extends to 3 mouse strains, our recent findings using thirteen HSV-2 *ts* mutants in the Biozzi strain [24].

MATERIALS AND METHODS

These are as described previously [24,25]. Mice of 3 strains bred on site were: Pirbright (a randomly mated closed colony), Biozzi (high antibody responder), and A.

We examined HSV-2 strain HG52 *wt* and 7 derived *ts* mutants from different complementation groups [21–23]. Aliquots (0.025 ml) of virus suspended in Dulbecco's phosphate-buffered saline supplemented with 10% calf serum were injected into the right rear FP of 3- to 4-week-old mice of either sex. The mouse core body temperature (38.5°C) conforms to nonpermissive temperature for the 7 *ts* mutants; however, when the mice are active, their FPs may be somewhat below the core body temperature, but when close packed and asleep in the nest, the FPs too will be at or near the core temperature. Mice were kept for at least 3 months following infection. None of the mice inoculated with HSV-2 strain HG52 *wt* or the *ts* mutants died as a consequence, though some developed temporary swelling and reddening of the injected FP lasting 2–3 days. Altogether 4 mice, all inoculated with *wt*, developed paralysis. Three months later, the mice were killed and tested for latent virus by immediately explanting the left and right DRG and left and right FP. Ganglia and FPs were placed individually into microtiter plate wells in medium consisting of Eagle's/50% fetal calf (EFC50) and incubated at 31°C for up to 60 days with screening of the supernatant daily (FP) or on alternate days (DRG) for infectious virus by plating onto monolayers of BHK21/C13 cells. To be scored as negative, DRG or FP had to have been incubated for a minimum of 21 days. All the isolates were checked for temperature sensitivity. Invariably, *ts* mutants retained their *ts* phenotype while *wt* virus remained non-*ts*.

FP tissue was explanted by first cleaning and washing both hind feet with absolute alcohol, then a sliver of FP was excised from each side with separate sterile instruments for each and dipped for 20 s into absolute alcohol. After washing the explanted tissue twice in EFC50, fragments were placed into separate microtiter wells, epidermal side up, and sufficient EFC50 added to cover the epidermal surface. Sensory ganglia (2 thoracic, the 6 lumbar, and the 1st sacral), were dissected out, washed separately, and placed into individual microtiter plate wells containing EFC50. Incubation proceeded at 31°C.

For dissociation of DRG, ganglia from the left and right sides were removed from pairs of mice and placed into 2 pools, one from the left and one from the right. The pooled ganglia were teased with fine forceps and then digested in 2 ml of 2% collagenase by incubation at 37°C for 2 h. Subsequently, the ganglia were dissociated into a single cell suspension by trituration through a plastic (Eppendorf) tip, centrifuged at 500 *g* for 10 min and resuspended in 15 ml fresh medium containing 5% human serum. Each cell suspension was then distributed in 3-ml volumes to 5 30-mm plastic Petri dishes (Nunc) containing a semiconfluent monolayer of BHK21/C13 cells. The dishes were then incubated at 31°C for 10 days, fixed and stained with Giemsa, and the number of infectious centers enumerated by scoring plaques.

On several occasions, DRGs and FPs were homogenized immediately following dissection and test-plated, but no infectious virus was ever detected.

RESULTS

Table I lists the recovery of 7 *ts* mutants and the *wt* of HSV-2 HG52 from DRG and FP of 3 mouse strains. At no time was virus recovered either from the uninoculated left rear FP or from the contralateral DRG. The infecting dose used lay between $1.2–17 \times 10^5$ pfu except for *ts*4 where it was 50×10^5 pfu. At the time of inoculation, the inoculum was simultaneously titrated on BHK21 C13 cells.

A number of points emerge:

1. *Wt* and *ts* mutant virus was recovered, not only from the DRG, but also from the FP tissue of all 3 mouse strains.
2. In general, *ts* mutants reactivated from the DRG less frequently than the *wt* virus. This trend is also noticeable for FP.
3. There is a (not always consistent) strain-dependent difference in the number of mice from which latent virus reactivates: the order is A > Biozzi > Pirbright.
4. With respect to latency, the *ts* mutants can be separated into 4 classes: *Class 1* behaves more or less like *wt* and can be recovered both from the DRG and from the FP

TABLE I. *Recovery of HSV-2 HG52 wt or ts virus from dorsal root ganglia (DRG) and footpad (FP) of 3 different strains of mice*

Virus	Dose (pfu × 10^5)	Mouse strain: Pirbright DRG	Pirbright FP	Biozzi DRG	Biozzi FP	A DRG	A FP	Total DRG	Total %	Total FP	Total %
*ts*2	17.0	0/14[a]	1/11[b]	8/17	1/16	8/9	2/9	16/40	40.0	4/36	11.1
*ts*3	1.2	0/14	1/6	0/16	5/15	0/24	5/15	0/54	0	11/36	30.5
*ts*4	50.0	2/15	0/15	12/14	1/14	6/15	8/14	20/44	45.5	9/43	20.9
*ts*5	17.0	0/13	0/13	0/12	0/12	0/11	0/11	0/36	0	0/36	0
*ts*11	4.2	0/12	0/12	1/12	0/12	2/13	0/12	3/37	8.1	0/36	0
*ts*12	5.0	3/14	1/12	0/14	1/14	4/13	1/11	7/41	17.1	3/37	8.1
*ts*13	6.0	2/11	1/9	2/14	0/14	1/11	2/11	5/36	13.9	3/34	8.8
Total *ts*		7/93	4/78	23/99	8/97	21/96	18/83	51/288		29/258	
% *ts* positive		7.5	5.1	23.2	8.2	21.9	21.7		17.7		11.2
wt HG52	1.0	9/15	3/22	11/13	1/14	5/5[c]	3/5[c]				
% *wt* positive		60	13.6	84.6	7.1	100	60				

[a] Number of positive/total mice tested.
[b] Despite precautions, the explanted FP tissue became contaminated in a number of cases and was discarded.
[c] 1.0×10^6 pfu/mouse.

(*ts*2, *ts*4, *ts*12, and *ts*13). *Class 2* is not recovered from either DRG or FP (*ts*5). *Class 3* is recovered only from the DRG and not from the FP (*ts*11). *Class 4* is recovered only from the FP but not from the DRG (*ts*3).

The number of mice of the 3 strains tested is just over 100 each and the virus has been tested only at a single dose to date. Thus when these investigations are extended, the class separation of the mutants' behavior may become less clearcut and "only rarely" may have to be substituted for "not" in the definitions. Nevertheless, we consider that our data are sufficient to justify the above assignment of our 7 mutants to the 4 classes. More extensive data may reveal more subtle *ts* mutant–mouse strain interactions, as possibly already suggested by *ts*2.

Table I shows that *wt* HG52 is readily recovered from latency in the DRG of all 3 strains of mice: 85% of the Biozzi strain and 60% of the Pirbright strain yielded virus after an initial inoculum of 1.0×10^5 pfu (injected with 1.0×10^6 pfu of *wt* virus, 6/6 of Biozzi and 6/6 of Pirbright strain mice yielded virus). Although the *wt* can be assumed to replicate readily at the peripheral and core temperatures of the mice, virus is much less often recovered from the FP than from the DRG of these 3 strains of mice.

Mutant *ts*2 behaved somewhat similarly to *wt* virus, but did not reactivate from the DRG of Pirbright mice (14 mice). Mutant *ts*3 was at no time recovered from the DRG but readily from the FP of all 3 strains of mice. Mutant *ts*4 behaved somewhat similarly to *wt* both for DRG and FP. This virus reactivated with very high efficiency from the DRG of Biozzi mice (12/14), but they had been injected at the high infectious dose of 5.0×10^6 pfu/mouse. Mutant *ts*5 has never been recovered from DRG or FP of the 3 mouse strains. Mutant *ts*11 has been recovered 3 times from DRG, (1/12 in Biozzi, 2/13 in A), but never from the FP. Mutants *ts*12 and *ts*13 behaved somewhat similarly to *wt*.

If we compare the combined results from the 6 reactivation positive mutants (*ts*2, 3, 4, 11, 12, and 13) with the *wt*, we find for the DRG 7/80 vs 9/15 (Pirbright); 23/87 vs 11/13 (Biozzi); 21/85 vs 5/5 (A), and for the FP 4/65 vs 3/22 (Pirbright); 8/85 vs 1/14 (Biozzi); and 18/72 vs 3/5 (A). Thus, on average, the *ts* mutants are much less often recovered from the DRG than are the *wt*, but are recovered with comparable frequency from the FP. These differences obtain despite much higher doses of *ts* mutant virus (in the cases of *ts*2, *ts*4, and *ts*5) than *wt* virus.

What is the evidence of virus latency in the FP, as opposed to persistent local infection at the site of inoculation?

First, on several occasions FPs have been homogenized immediately following dissection and screened for virus—always with negative results.

Second, we have data for 37 mice (4 of these had been inoculated with HG52 *wt* and 33 with intertypic HSV-1/HSV-2 recombinants which grow normally at 31°C or 38.5°C) where a FP was cut in half and one piece immediately homogenized while the other was explanted and incubated as usual. Virus could in no case be recovered from the homogenized half, but reactivation took place after several days in each of the 37 explanted half FPs.

Third, the time of first virus recovery from FP indicates latency (Table II). The earliest observation of reactivation from the DRG has been on day 8 after explantation, while in some cases virus was not detected in the supernatant before day 26. The respective FP data showed the earliest time of virus detection to be day 8 and the latest day 44 following explantation. Thus the FP mode of reactivation closely reflects reactivation from latency in DRG, but does not conform with the rapid recovery expected were the FP persistently infected. It is worth pointing out that the time of reactivation from the explants does not appear to vary among mouse strains.

We have obtained some evidence concerning the effect of varying the virus dosage at the time of infection on the ability to reactivate latent HSV-1 strain 17 *wt* virus from the DRG of Biozzi mice. The results in Table III show the average number of infectious centers per mouse obtained from plating out dissociated ganglia. The number of foci (infectious centers) recoverable from dissociated DRG increases with the dose initially used to infect them, ranging from an average of 4 foci per mouse at a dose of 3.0×10^3 pfu to 85 foci per mouse at 1.0×10^7 pfu. Following infection with high doses, virus has also been reactivated from the contralateral ganglia but at much lower efficiency (Table III).

DISCUSSION

Over recent years, opinion has hardened that HSV latency is due to the viral genome entering the still undefined dormant state in the neuronal cell body in sensory ganglia. The most direct evidence is that HSV antigens first appear in neurons when DRG of latently infected mice are undergoing reactivation in vitro [25–27]. In humans, with one possible exception [28], HSV has only been reactivated in culture from the latent state in tissue in which neuronal cell bodies are known to be present. It has, however, been known and accepted for many years now—largely due to the work of Scriba—that HSV-2 can be recovered from what appeared to be a persistent infection at the site of inoculation in the FP of the guinea pig. Unlike the situation we have found in the mouse, infectious virus could be demonstrated frequently in the guinea pig FP soon after explantation [12–14]. HSV-2 has been isolated also by another group of investigators from the guinea pig FP [29], and HSV-1 from the ear skin (the site of inoculation) in 8% of latently infected mice, in the absence of observable lesions [30]. In general, the view that the neuronal cell body is where virus establishes latency has become so entrenched that the possi-

TABLE II. *Time of reactivation of virus from dorsal root ganglia (DRG) and footpad (FP) in different strains of mice*

Virus	Mouse strain					
	Pirbright		Biozzi		A	
	DRG	FP	DRG	FP	DRG	FP
*ts*2	–	44	17–23 (19.2)[a]	23	14–16 (15.2)	17–21 (19)
*ts*3	—	34	—	10–25 (15.8)	—	14–16 (14.6)
*ts*4	20	—	10–20 (16.2)	37	11–16 (13.1)	12–19 (15.8)
*ts*5	—	—	—	—	—	—
*ts*11	–	—	14	—	14–25 (19.5)	—
*ts*12	17	8	—	23	10–16 (14)	15
*ts*13	14–18 (16)	20	11–14 (12.5)	—	18–24 (22)	13–18 (15.5)
wt	8–26[b] (15.8)	18–24[b] (22)	10–26[b] (21.1)	43[b]	10–15[c] (11)	10–27[c] (15.6)

[a] Figure in parenthesis indicates the mean.
[b] 1.0×10^5 pfu/mouse.
[c] 1.0×10^6 pfu/mouse.

TABLE III. *Infectious center assay of cells from dissociated Biozzi ganglia latently infected with HSV-1 wt*

	Dose of virs inoculated (pfu)			
	3.0×10^3	2.0×10^5	5.0×10^5	1.0×10^7
Right DRG (side of infection)	4[a]	21	38	85
Left DRG	0	0	3	15

[a] Number of foci of cytopathic effects (cpe) scored on an indicator layer of BHK21 C13 cells, each number is the average of 4 mice.

bility that HSV might achieve latency in other cell types has largely been discounted.

Three different models of virus latency have been proposed. Basing his own seminal findings on much earlier but less critical work, Stevens developed the classical hypothesis of latency [7]: this proposes that the viral genome usually remains in a dormant state in the neuronal cell body, but is from time to time induced to reactivate, producing virus particles which travel down the axon and then infect other cell types after release from nerve fibrils in the skin. A second hypothesis, the "skin trigger hypothesis" of Hill and Blyth [31], proposes latency to be a dynamic state undergoing a continuing series of reactivations which most of the time, however, only give rise to subclinical infections or abort at the periphery. Events in the skin itself trigger and primarily determine whether a particular episode of reactivation will succeed in producing detectable lesions at the periphery. A third hypothesis, "the round trip," was put forward by Klein [32]. This proposes that the reactivated virus moves from the neuronal cell body down the axon to infect cells at the periphery, the infected neurons in the process being destroyed; during amplification, the released virus infects neighboring fibrils of other neurons and travels up the axon to establish latency in their cell bodies. Thus the numbers of latently infected neurons are maintained in the individual. At present there is no final consensus of opinion, but it will be noted that the three hypotheses are not necessarily mutually exclusive. All three hypotheses involve the neuronal cell body as an essential component. None addresses itself to the problem which our investigations with HSV-1 [33] and HSV-2 [24] have raised, namely, that virus appears to be able to attain a state in FP tissue that is indistinguishable from latency. We are not aware of any anatomic evidence to suggest that nucleated neuronal cell bodies are likely to be present in mouse FP tissue, though it must be stressed that the explanted tissue stems from crude dissection of skin slivers and probably contains additional deeper dermal tissues as well. Latent HSV clearly cannot maintain itself by replicating because antiviral drugs (like acyclovir) appear to be unable to eradicate the latent virus [34]. We propose that there are cell types other than neurons which HSV can latently infect. Some of our future experiments will be directed toward identifying these cell types.

Virus reactivated at different frequency from mice of 3 different strains that had been injected with the same virus dose [35,36]. This indicates that the host's genetic makeup has an important influence on the outcome. The effect of mouse host genetic makeup on HSV pathogenicity has been elegantly studied [37,38], but at present a similar detailed study vis-à-vis latency remains to be undertaken. The present data and results obtained by us with HSV-1 and 5 mouse strains [36] do not suggest a correlation between mouse strain susceptibility to HSV-1 or HSV-2 and ability to act as a host for latent virus. It is relevant in this context that HG52 is unusual among HSV-2 strains in being of very low pathogenicity.

Previously the only viral gene recognized to have a function necessary for latency was the HSV thymidine kinase gene [39]. Our experiments have produced clear evidence that latency is dependent on several viral gene functions, some of which we have pinpointed by *ts* mutations in different HSV-2 genes. The generally lower rate of successful establishment of latency by *ts* mutants is notable, although it may reflect inability to replicate efficiently following injection. The real interest lies in the identification of mutants like *ts*5, *ts*3, and *ts*11. *Ts*5 seems to preclude HSV latency in either DRG or the FP of the mouse strains—clearly an interesting mutant to investigate in more detail and such studies are under way. *Ts*3 is also of special interest, being recovered only from explanted mouse FP. *Ts*11 based on more limited data appears to have the opposite capability, being recovered only from the DRG. These two mutants imply differences in the molecular events involved in the process of establishing latency at the two different sites. It will be of considerable interest and importance to identify and characterize the relevant gene products and attempt to study their biological functions. It is of particular interest that mutants *ts*3 and *ts*5 are DNA-positive, while *ts*11 is DNA-negative. It should be mentioned that *ts*3 could not be recovered even after dissociation of the DRG of 4 A strain mice (data not shown).

We draw attention to the results obtained with dissociated DRG from mice infected initially with different doses of HSV-1. The number of foci observed in the monolayers gives an estimate of the number of DRG cells from which HSV reactivates. These figures are likely to be underestimating the original number of neurons present from which HSV reactivates owing to loss of cells during dissociation and plating. We have shown that focus number increases with the initial dose and that given

5.0×10^5 pfu, virus spread becomes sufficiently extensive to allow the establishment of latency in the contralateral DRG of Biozzi mice. This suggests that the initial infecting dose used can importantly influence the outcome with respect both to frequency and extent of the establishment of latency.

Finally, we believe our data on the mouse FP necessitate reinvestigation of the situation in humans. In particular, we stress the importance that explanted tissue containing both epidermis and dermis be incubated, constantly monitored, and followed for a sufficient time to ensure the validity of negative results. For example, our investigations in the FP of mice with HSV-2 would not have yielded any positive data had the experiments been terminated before the eighth day. In the great majority of cases, successful virus isolation did not occur until much later.

It is a pleasure to thank Mrs. A. J. Simpson for her patience in typing the manuscript, and Miss M. Braidwood for technical assitance.

REFERENCES

1. Wildy P, Field HJ, Nash AA: Classical herpes latency revisited, Virus Persistence. Edited by BWJ Mahy, AC Minson, GK Darby. New York, Cambridge Univ Press, 1982, pp 133–167
2. Bastian FO, Rabson AS, Yee CL, Tralka TS: *Herpesvirus hominis*, isolation from human trigeminal ganglia. Science 178:306–307, 1972
3. Warren KG, Brown SM, Wroblewska Z, Gilden D, Koprowski H, Subak-Sharpe JH: Isolation of latent herpes simplex virus from the superior cervical and vagus ganglions of human beings. N Engl J Med 298:1068–1069, 1978.
4. Spruance SL, Overall JC Jr, Kern ER, Krueger GG, Pliam V, Miller W: The natural history of recurrent herpes simplex labialis. N Engl J Med 297:69–74, 1977
5. Baringer JR: Recovery of herpes simplex virus from human sacral ganglions. N Engl J Med 291:828–830, 1974
6. Kitchener HC, Cordiner JW, Eglin RP: Latency of herpes simplex virus in uterosacral ligaments. Am J Obstet Gynecol 43:839–840, 1982
7. Stevens JG: Latent herpes simplex virus and the nervous system. Curr Top Microbiol Immunol 70:31–50, 1975
8. Rustigan R, Smulow JB, Tye M, Gibson WA, Skindell E: Studies on latent infection of skin and oral mucosa in individuals with recurrent herpes simplex. J Invest Dermatol 47:218–221, 1966
9. Lonsdale DM, Brown SM, Subak-Sharpe JH, Warren KG, Koprowski H: The polypeptide and the DNA restriction enzyme profiles of spontaneous isolates of herpes simplex virus type 1 from explants of human trigeminal, superior cervical and vagus ganglia. J Gen Virol 43:151–171, 1979
10. Lonsdale DM, Brown SM, Lang J, Subak-Sharpe JH, Koprowski H, Warren KG: Variation in HSV isolated from human ganglia and a study of clonal variation in HSV-1. Ann NY Acad Sci 354:291–308, 1980.
11. Davison AJ, Wilkie NM: Nucleotide sequences of the joint between the L and S segment of herpes simplex virus types 1 and 2. J Gen Virol 55:315–331, 1981
12. Scriba M: Recurrent genital herpes simplex virus (HSV) infection of guinea pigs. Med Microbiol Immunol (Berl) 162:201–208, 1976
13. Scriba M: Extraneural localization of herpes simplex virus in latently infected guinea pigs. Nature 267:529–531, 1977
14. Scriba M: Persistence of herpes simplex virus (HSV) infection in ganglia and peripheral tissues of guinea pigs. Med Microbiol Immunol (Berl) 169:91–96, 1981
15. Hill TJ, Field HJ, Blyth WA: Acute and recurrent infection with herpes simplex virus in the mouse: a model for studying latency and recurrent disease. J Gen Virol 28:341–353, 1975
16. Blyth WA, Hill TJ, Field HJ, Harbour DA: Reactivation of herpes simplex virus infection by ultraviolet light and possible involvement of prostaglandins. J Gen Virol 33:547–550, 1976
17. Hill TJ, Blyth WA, Harbour DA: Trauma to the skin causes recurrence of herpes simplex in the mouse. J Gen Virol 39:21–28, 1978
18. Harbour DA, Hill TJ, Blyth WA: Recurrent herpes simplex in the mouse: inflammation in the skin and activation of virus in the ganglia following peripheral stimulation. J Gen Virol 64:1491–1498, 1983
19. Lofgren KW, Stevens JG, Marsden HS, Subak-Sharpe JH: Temperature-sensitive mutants of herpes simplex virus differ in the capacity to establish latent infection in mice. Virology 76:440–443, 1977
20. Watson K, Stevens JG, Cook ML, Subak-Sharpe JH: Latency competence of thirteen HSV-1 temperature-sensitive mutants. J Gen Virol 49:149–159, 1980
21. Timbury MC: Temperature-sensitive mutants of herpes simplex virus type 2. J Gen Virol 13:373–376, 1971
22. Halliburton IW, Timbury MC: Characterization of temperature-sensitive mutants of herpes simplex virus type 2. Growth and DNA synthesis. Virology 54:60–68, 1973
23. Halliburton IW, Timbury MC: Temperature-sensitive mutants of herpes simplex virus type 2: description of three new complementation groups and studies on the inhibition of host cell DNA synthesis. J Gen Virol 30:207–221, 1976
24. Al-Saadi SA, Clements GB, Subak-Sharpe JH: Viral genes modify herpes simplex virus latency both in mouse footpad and sensory ganglia. J Gen Virol 64:1175–1179, 1983
25. Kennedy PGE, Al-Saadi SA, Clements GB: Reactivation of latent herpes simplex virus from dissociated identified dorsal root ganglion cells in culture. J Gen Virol 64:1629–1635, 1983
26. McLennan JL, Darby G: Herpes simplex virus latency: the cellular location of virus in dorsal root ganglia and the fate of the infected cell following virus activation. J Gen Virol 51:233–243, 1980
27. Cook ML, Bastone VB, Stevens JG: Evidence that neurones harbour latent herpes simplex virus. Infect Immun 9:946–951, 1974
28. Shimeld C, Tullo AB, Easty DL, Thomsitt J: Isolation of latent herpes simplex virus from the cornea in chronic stromal keratitis. Br J Ophthal 66:643–647, 1982
29. Donnenberg AD, Chaikoff E, Aurelian L: Immunity to herpes simplex virus type 2: cell-mediated immunity in latently infected guinea pigs. Infect Immun 30:99–109, 1980
30. Hill TJ, Harbour DA, Blyth WA: Isolation of herpes simplex virus from the skin of clinically normal mice during latent infection. J Gen Virol 47:205–207, 1980
31. Hill TJ, Blyth WA: An alternative theory of herpes simplex recurrence and a possible role for prostaglandins. Lancet 1:397–399, 1976
32. Klein RJ: Pathogenetic mechanisms of recurrent herpes simplex virus infections. Arch Virol 51:1–13, 1976
33. Subak-Sharpe JH, Al-Saadi SA, Clements GB: Herpes simplex virus type 1 and 2 genes affecting latency in mouse footpad and sensory ganglia, Latent Herpes Virus Infections in Veterinary Medicine. Edited by G Wittmann, RM Gaskell, H-J Rziha. The Hague, Martinus Nijhoff, 1984, pp 63–72
34. Field HJ, Bell SE, Elion GB, Nash AA, Wildy P: Effect of acycloguanosine treatment on acute and latent herpes simplex infections in mice. Antimicrob Agents Chemother 15:534–561, 1979
35. Harbour DA, Hill TJ, Blyth WA: Acute and recurrent herpes simplex in several strains of mice. J Gen Virol 55:31–40, 1981
36. Clements GB, Subak-Sharpe JH: Recovery of herpes simplex virus type 1 *ts* mutants from the dorsal root ganglia of mice. Prog Brain Res 59:203–208, 1983 Published by Elsevier.
37. Lopez C: Genetics of natural resistance to herpes virus infections in mice. Nature 258:152–153, 1975
38. Kirchner H, Hochen M, Hirt HM, Munk HZ: Immunological studies of HSV infection of resistant and susceptible inbred strains of mice. Zeitschrift fur Immunitatsforschung und Experimentelle Therapie 154:147–154, 1978
39. Field HJ, Wildy P: The pathogenicity of thymidine kinase-deficient mutants of herpes simplex virus in mice. J Hyg (Cambridge) 81:267–277, 1978